Escherichia coli cells, showing improved definition of cell boundaries and subcellular features. Our studies provide a simple method to acquire the PSF, making possible the extension of deconvolution beyond fluorescence to include bright field as well.

4015-Pos Board B743

Fast Binding Kinetics of RNA Aptamers Measured using a Novel Microfluidic Mixer

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RNA aptamers that target specific biomolecules with high specificity are growing in popularity due to their ease of production compared to antibodies and their tight binding. Aptamers have uses ranging from targeted protein inhibition to the creation of new fluorescent labels. One example of the latter is the Spinach aptamer which binds 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI) to form a fluorescent complex resembling the chromophore of GFP. Upon binding its target, the RNA aptamercauses a conformational stabilization of DFHBI resulting in a fluorescent complex. The kinetics of Spinach aptamer binding and activation of the fluorophore have not been thoroughly investigated. Using a novel microfluidic fast mixing device which has been shown to have dead time for mixing of ~500 μs and be especially useful for viscous solutions, we have examined the on-rate of the Spinach/DFHBI binding reaction to gain an increased understanding the kinetics of aptamer-ligand binding.

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Development of a Slow-Switching Dronpa Variant for 2-Color Super Resolution Imaging of Drp1 During Mitochondrial Fission

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We studied the single-molecule photo-switching properties of Dronpa, a green photo-switchable fluorescent protein (PS-FP) and a popular marker for photo-activated localization microscopy (PALM). We found the excitation light photo-activates as well as deactivates Dronpa single molecules, hindering temporal separation and limiting super resolution. To resolve this limitation, we have developed a novel Dronpa variant, rsKame. The increased steric hindrance generated by the mutation reduced the excitation light-induced photo-conversion from the dark to fluorescent state. To demonstrate the applicability of rsKame, we paired it with PAmCherry1 in a 2-color PALM imaging method to observe the inner and outer mitochondrial membrane structures and selectively labeled dynamin related protein 1 (Drp1), responsible for membrane scission during mitochondrial fission. We quantified Drp1 helical ring diameters and lengths showing that the rings undergo changes in diameter between different stages of mitochondrial fission without significant modification of their length. These results support the twistase model of Drp1 constriction with some loss of subunits at the helical ends.

4017-Pos Board B745

Wide-Field Background Free Imaging by Magnetic Modulation of Nanodiamond Fluorescence

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Autofluorescence and other sources of background noise often limit fluorescence based imaging, particularly in vivo. To overcome these limitations, we developed a wide field background-free imaging technique based on magnetic modulation of the emission of fluorescent nanodiamonds combined with phase-sensitive detection. Negatively charged nitrogen-vacancy (NV) centers in fluorescent nanodiamonds are fluorescent sources with remarkable optical properties. They do not photo-bleach or blink and their fluorescence emission can be modulated with a magnetic field. NV^- centers have broad excitation (~500-600 nm) and emission (~600-700 nm) spectra and the red to near infrared emission facilitates in vivo detection. We demonstrate in vitro and in vivo background-free imaging through the application of an alternating magnetic field during imaging with a conventional fluorescence microscope. Post-processing of the images to extract the modulated signal from the background improves the signal-to-noise ratio by as much as 100-fold over conventional florescence imaging techniques. As a proof of principle, we demonstrate in vivo background-free detection of ~100 nm fluorescent nanodiamonds taken up by primary draining lymph nodes in mice. Background-free imaging of NV^- centers is readily implemented on any imaging platform by combining magnetic field modulation with straightforward image processing. We present two alternative image processing approaches to improve the signal-to-noise of the magnetically modulated fluorescence images.

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A Spectral Phasor Perspective in Zebrafish Muscle Development

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Hyperspectral imaging provides the potential for assessing biochemical interactions in the zebrafish embryo in a label-free manner that extends beyond conventional morphological and molecular phenotyping. It takes advantage of the intrinsic wavelengths emitted or reflected from a sample without the need for extrinsic staining methods. The specific spectral signature from a sample can arise from chemical interactions, molecular bonds and macrostructural arrangements. A challenge in hyperspectral imaging is the large spectral data sets that result from acquiring a spectrum for every pixel within an image. Spectral Phasor offers an efficient representation of the spectral data as vectors in Fourier space, thereby condensing each spectrum into a single point in a 2-D plot. The Spectral Phasor has been successfully applied to hyperspectral data on protein samples, demonstrating changes in fluorescence signatures. This study proposes an application of Spectral Phasor to the zebrafish muscle development. The skeletal muscle system provides an attractive model for the proof-of-principle experiments in the implementation of Spectral Phasor. Skeletal muscle is a highly organized tissue with myofibrils as the functional unit that contributes to the repetitive segment of the myotome. The modularity of these units provides unique landmarks for anchoring the SP data. Our analysis of muscle suggest that SP can be used for staging the skeletal muscle development.

4019-Pos Board B747

Quantifying Protein Conformation Heterogeneity in Live Cells by Fourier Lifetime Excitation-Emission Matrix Spectroscopy

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FLIM-FRET is a powerful imaging technique to study molecular interactions and conformational changes in biological systems. However, current FLIM-FRET techniques have difficulties to provide robust quantitative analysis of heterogeneous protein species due to several factors arising from live cell imaging, including low signal to noise ratio, small lifetime differences and intensity imbalance between high and low FRET species. Here we present a FRET lifetime analysis method based on frequency domain Fourier lifetime excitation-emission matrix (FLEEM) imaging. FLEEM is a novel high-speed multiplexed frequency-domain lifetime imaging method developed by our group. With FLEEM, time-resolved fluorescence images of live cells can be acquired simultaneously in multiple excitation-emission channels, including the donor spontaneous emission, acceptor spontaneous emission induced by direct acceptor excitation and the sensitized emission from the acceptor induced by FRET[1]. Assuming a two conformation model, each protein conformation has a distinct FRET efficiency, which results in two distinct donor lifetimes. The measured average donor lifetimes in both donor spontaneous emission and FRET-induced sensitized emission channel are population weighted averages of these two lifetimes, readily described by a two-dimension linear parametric function, of which weighting coefficients of two lifetime species differ between two channels. Two FRET efficiencies corresponding to low and high FRET conformation can be extracted from parametric fitting of the experimentally measured life times. The ratio of the two conformation populations can then be easily computed from the average donor